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Symmetry and Chirality in Topoisomerase II-DNA Crossover Recognition

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Several experimental data support the notion that the recognition of DNA crossovers play an important role in the multiple functions of topoisomerase II. Here, a theoretical analysis of the possible modes of assembly of yeast topoisomerase II with right and left-handed tight DNA crossovers is performed, using the crystal coordinates of the docking partners. The DNA crossovers are assumed to be clamped into the central hole of the enzyme. Taking into account the rules for building symmetric ternary complexes and the structural constraints imposed by DNA-DNA and protein-DNA interactions, this analysis shows that two geometric solutions could exist, depending on the chirality of the DNA crossovers. In the first one, the two DNA segments are symmetrically recognized by the enzyme while each single double helix binds asymmetrically the protein dimer. In the second one, each double helix is symmetrically recognized by the protein around its dyad axis, while the two DNA segments have their own binding modes. The finding of potential DNA-binding domains which could interact with the crossovers provides structural supports for each model. The structural similarity of a loop containing a cluster of conserved basic residues pointing into the central hole of topoisomerase II and the second DNA-binding site of histone H5 which binds DNA crossover is of particular interest. Each solution, which is consistent with different sets of experimental data found in the literature, could either correspond to different functions of the enzyme or different steps of the reaction. This work provides structural insights for better understanding the role of chirality and symmetry in topoisomerase II-DNA crossover recognition, suggests testable experiments to further elucidate the structure of ternary complexes, and raises new questions about the relationships between the mechanism of strand-passage and strand-exchange catalyzed by the enzyme.

Keywords: recombination; DNA-binding protein; cruciform; DNA structure; DNA condensation

The dual catalytic and structural role of type II DNA topoisomerase is essential for the survival of eukaryotic cell (Osheroff *et al*., 1991; Chen & Liu, 1994; Wang, 1996). Several lines of experimental evidences suggest that DNA topoisomerase II exerts its enzymatic and structural functions by recognizing DNA crossovers. Indeed, electron microscopic studies have shown that the enzyme can recognize DNA crossovers or the base of DNA loops (Zechiedrich & Osheroff, 1990; Howard *et al*., 1991; Howard & Griffith, 1993). The dependence of a second double helix for strand cleavage (Corbett *et al*., 1992) and the requirement of topoisomerase II during anaphase (Jannink *et al*., 1996; Sikorav *et al*., 1998) have indicated that binding DNA crossovers could play a role in the strand-passage reaction. The implication of type II topoisomerase in illegitimate recombination (Sperry *et al*., 1989; Bae *et al*., 1988), in SV40 integration (Bodley *et al*. 1993), its ability to perform intermolecular ligation (Gale

Abbreviations used: HTH, helix-turn-helix.

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& Osheroff, 1992; Schmit *et al*., 1994) and cleavage of DNA hairpin (Froelich-Ammon *et al*., 1994) suggests that the enzyme could act on DNA synaptic structures in a manner similar to recombinase enzymes. In other respects, as many other crossover-binding proteins involved in the organization of DNA such as histone H1, HMG and HU (Bianchi *et al*., 1989; Krylov *et al* (1993); Varga-Weisz *et al*., 1994; Bonnefoy *et al*., 1994; Pontiggia *et al*., 1993), topoisomerases II contribute to the condensation of higher-order DNA structures (Berrios *et al*., 1985; Gasser & Laemmli, 1986; Adachi *et al*., 1989). It is thought that the enzyme fastens the chromosomal loops of the metaphase chromosomes in binding to DNA crossovers.

Solving the structure of a ternary complex topoisomerase II-DNA crossover is, therefore, an indispensable step in elucidating how topoisomerase II exerts its multiple functions. Although the crystal structures of the large fragments of eukaryotic and prokaryotic enzyme have provided significant insights for better understanding the enzymatic mode of action (Berger *et al*., 1996; Morais Cabral *et al*., 1997), the mechanism of strand-passage reaction is still not completely understood. In particular, the role of DNA crossover is controversial (Roca & Wang, 1992; Chen & Liu, 1994; Maxwell, 1996). Moreover, several structural problems related to the simultaneous binding of two DNA segments on the protein remain to be elucidated. It is not known, for example, how topoisomerase II can distinguish right-handed from left-handed crossovers (Roca & Wang, 1996; Shaw & Wang, 1997). Another question is why the enzyme does not bind to and cleave a symmetric consensus sequence (Sander & Hsieh, 1985), as other protein homodimers which recognize symmetrically palindromic DNA duplexes around their dyad axes. The problem of symmetry in topoisomerase II-DNA recognition is further complicated by the observation that topoisomerase II is able to discriminate and to cleave preferentially one strand of the DNA double helix (Muller *et al*., 1988; Andersen *et al*., 1989; Zechiedrich *et al*., 1989).

Current models using the crystal structures of eukarytotic and prokarytic enzyme suggest the binding of a single DNA segment into a cleft having a strong positive electrostatic potential and containing the helix-turn-helix motif (Berger *et al*., 1996; Morais Cabral *et al*., 1997). An alternative view is that one or two DNA segments can be clamped into the central hole of the enzyme at the interface between the two monomers (Roca & Wang, 1992; Chen & Liu, 1994; Timsit & Moras, 1994; Maxwell, 1996). However, little is known about the details of this type of interaction. Here the binding of two DNA segments into the large hole of the yeast enzyme (Berger *et al*., 1996) is investigated in the light of crystallographic studies of right and left-handed tight DNA crossovers (Timsit & Moras, 1996; Timsit *et al*., 1998). The crystal structures of the docking partners have been used for analyzing the possible modes of assembly of symmetric ternary complexes taking into account the constraints imposed by the chirality and symmetry of DNA crossovers. DNA-protein interactions were modelled when considering the existence of potential DNA-binding domains located around the central hole of the yeast enzyme. These domains were identified on the basis of their structural similarity with DNA-binding motifs found in the literature. The functional significance of these assemblies is discussed in the light of experimental data of the literature.

Structural properties of right and left-handed DNA crossovers

Groove-backbone interaction imposes the geometry and the chirality of self-fitted DNA duplexes and produces 2-fold symmetric right-handed DNA crossovers (Timsit *et al*., 1989; Timsit & Moras, 1991, 1994; Table 1). Biochemical studies have shown that Holliday junctions can adopt similar

Table 1. Structural properties of right and left-handed DNA crossovers and geometry of topoisomerase II crossover assembly

DNA crossover		
Chirality	Right-handed	Left-handed
Symmetry	2-fold symmetry 2-fold axis bisecting the Large angle (a1)	222 symmetry 2-fold axis bisecting the Large (a1) and small (a2) angles 2-fold axis perpendicular to the plane of the cross (a3)
Geometry		
Assembly	Groove-backbone	Major groove-major groove
Large angle size (deg.)	106	120
Interpenetration (Å)	5	2
Models	G1	G2
Correspondence of the 2-fold axes	Protein dimer – (a1)	Protein dimer – (a3)
Ternary complex symmetry	The two DNA segments are equivalents	The two DNA segments are not equivalent (N and C gate duplexes)
	Asymmetric binding of each DNA segment	Symmetric binding of each DNA segment

X-shaped right-handed DNA crosses in high salt conditions (Lilley & Clegg, 1993). The two DNA segments are related by a 2-fold axis (a1) which bisects the large angle of the crossover (Figure 1(a)). The sliding of the helices relatively to each other required for their mutual fit, prevents the pseudo-dyad axes to colineate at the intersection point of the cross. DNA self-fitting can trigger important alteration of DNA secondary structure in a sequence dependent manner such as the premelting of $(CA)_n$ repeats and $(C/A)_n$ sequences (Timsit *et al*., 1991; Timsit & Moras, 1995).

Another mode of close helical assembly producing left-handed DNA crossover was recently observed in decamer duplex crystal structures (Timsit & Moras, 1994; Shatzky-Schwartz *et al*., 1997; Y. Timsit *et al*., unpublished). Since right and left-handed crossovers are obtained in similar crystallization conditions, it seems likely that the oligonucleotide sequence has influenced the mode of DNA crossing (Y. Timsit *et al*., unpublished). Highly symmetric crossovers are produced when the major grooves fit together at the crossing point. The backbones of one helix are adjusted lengthwise along the helical axis of the other one, thus minimizing the repulsion of the negatively charged backbones. In contrast to right-handed crossovers, the pseudo-dyad axes of the two DNA segments are colinear at the crossing point. The resulting structure is, therefore, characterized by a 222 symmetry with three orthogonal 2-fold axes (Table 1). The 2-fold axes (a1) and (a2) bisect the large and the small angle, respectively. The third axis (a3) which corresponds to the colinear dyad axis of each helix is perpendicular to the plane of the cross (Figure 1(b)).

Viewing down the 2-fold axis (a1) of the right-handed crossovers, the two large angles are structurally distinct and suitable for the symmetric recognition by protein dimers, with the correspondence of the 2-fold axes of the protein and the cross (Figure 1(a)). The surface of the two small angles which are equivalent with the 2-fold axis (a1) is asymmetric. A different situation is observed for the left-handed crossovers. Due to the 222 symmetry, both large and small angles exhibit a symmetric surface and are structurally equivalent to each other, respectively (Table 1). The presence of a third 2-fold axis (a3) perpendicular to the plane of the cross, provides a new mode of symmetric recognition. Within the cross, the two helices can, therefore, be recognized symmetrically around their dyad axes in the classic manner of a protein dimer bound to a palindromic target sequence, while keeping the 2-fold symmetry of the overall crossover-protein complex.

Geometric solutions for topoisomerase II-DNA crossover assemblies

The formation of symmetric ternary complexes between the large fragment of yeast topoisomerase II and the right or left-handed DNA crossovers is analyzed, assuming that the cross is encircled by the enzyme ring at its intersection point in such manner that the 2-fold axis of the protein dimer corresponds with one 2-fold axis of the cross.

G1 geometry

When the 2-fold axis (a1) bisecting the large angle of right-handed DNA crossover matches with the axis of the protein dimer, the two DNA segments are symmetrically related and interact in a equivalent manner with the protein dimer (Figure 2(a)). In contrast, each double helix interacts asymmetrically with the enzyme, with respect to the 2-fold axis of the protein dimer (Table 1). One double helix passes from one protein monomer to the other one, in going from the top (N-gate) to the bottom (C-gate), and contacting consecutively the B′ and the A′ subfragments. The two large angles point towards the top and the bottom of the enzyme, while the small angles are opened towards the solvent (Figure 2(b)). The angle pointing towards the C-gate remains largely exposed to the solvent, while its arms are gripped symmetrically by two claws consisting of the anti-parallel β-sheets, β5-β6 and β17-β18, one helical turn away from the crossing point. The bottom of the small angle contacts a cluster of basic residues (Lys712, Lys713, Lys716 and Lys720) located on α1-β1-α3 (Table 2; Figure 3, see below). It should be noted that the G1 solution can be compatible with the model proposed by Berger *et al* (1996) if the DNA segments are bent for fitting into the cleft containing the helix-turn helix motif (Figure 2(c)). In a manner similar to that of right-handed crossover, the correspondence of the 2-fold axis of the protein and the 2-fold axis (a1) of a left-handed crossover can generate a symmetric ternary complex. In this case, however, the DNA segments cannot fit into the two β-sheet claws within the central hole and for sterical reasons, the surface of DNA-protein interaction is significantly reduced (results not shown).

G2 geometry

Another mode of assembly is produced when the third 2-fold axis (a3) of the left-handed crossover matches with the 2-fold axis of the protein homodimer (Figure 2(d)). In contrast with the G1 symmetry, the 2-fold axis of the protein dimer is perpendicular to the plane of the cross. In consequence, each DNA segment contacts symmetrically the two monomers around its dyad axes, in the classical manner of the symmetric recognition of a palindromic sequence. In contrast, the two DNA segments have their own mode of recognition. The N-gate helix passes close to the active site and is less protected than the C-gate helix. DNA bending makes possible the fitting of its two terminal parts into the cleft described by Berger *et al*. 19966; result not shown). The C-gate helix is gripped by the two antiparallel β-sheets claws and abut onto α17 (Figure 2(e), Table 2).

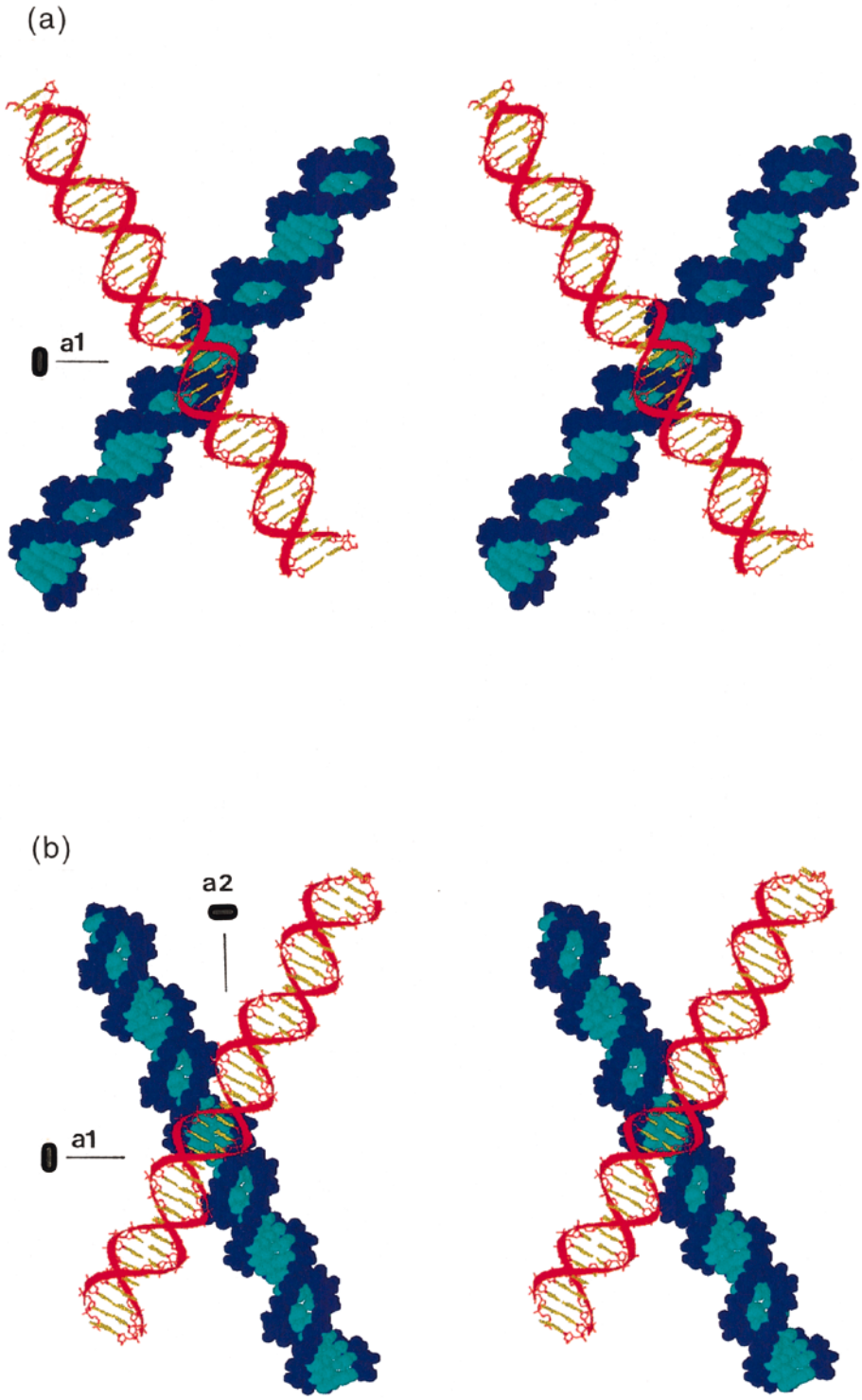

Figure 1. Geometry of right and left-handed DNA crossovers. (a) Stereo view of a right-handed NA crossover as found in the rhomboedral crystal packings of DNA duplexes. The 2-fold axis which bisects the large angle of the cross (a1) is indicated. (b) Stereo view of a left-handed crossover as found in the trigonal packing of the decamer duplex d(CCIIICCCGG). The three orthogonal 2-fold axes are indicated. The 2-fold axes (a1) and (a2) bisect the large and the small angle, respectively. The 2-fold axis (a3) is perpendicular to the two first ones and corresponds with the two dyad axes of each double helix at the intersection point. Idealized right and left-handed DNA crosses were generated by superimposing fiber coordinates of two *B*-DNA segments on symmetry related duplexes within the crystal packing of the dodecamer d(ACCGGCGCCACA) (Timsit *et* al., 1989) and the decamer d(CCIIICCCGG) (Shatzky-Schwartz *et al.*, 1997; Y. Timsit *et al.*, unpublished)), respectively. The crystals were grown in conditions usual for *B*-DNA duplexes with spermine/DNA and Mg/DNA stoechiometric ratio comprised between 1–2 and 7–20, respectively (Timsit & Moras, 1992). For commodity, the plane of a symmetric DNA crossover is defined as the plane located at the interface of the two DNA segments which is parallel to the two helical axes. This plane contains the 2-fold axis bisecting the large angle of the cross.

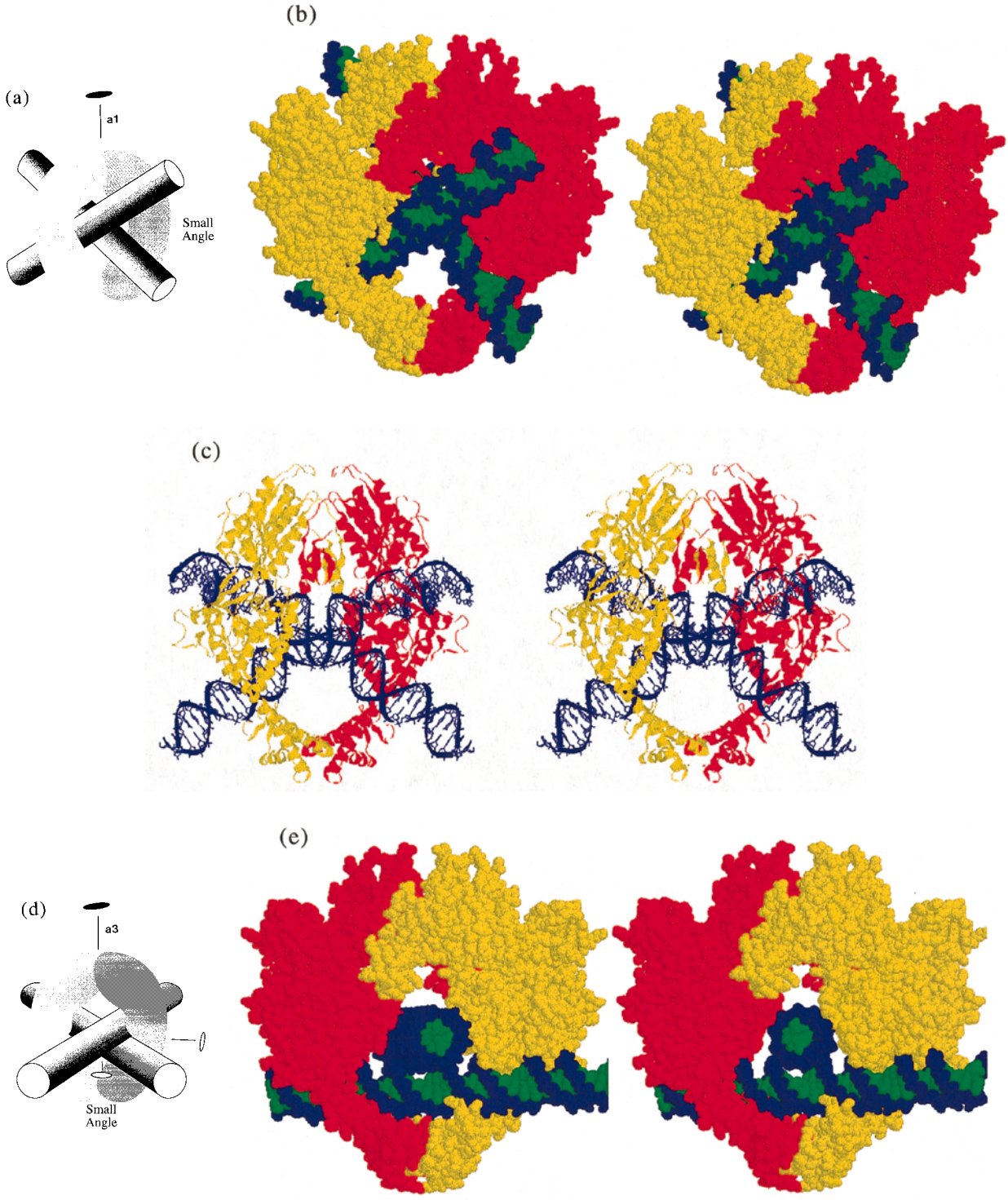

Figure 2. The two modes of symmetric assembly of topoisomerase II onto DNA crossovers. (a) and (b) Schematic representation and stereo views of the models in the G1 geometry. The enzyme is bound to a right-handed DNA crossover. The 2-fold axis of the protein dimer corresponds with the 2-fold axis (a1) of the crossover. (c) Stereo view of the ternary complex in G1 geometry in which the DNA segments are bent for fitting into the cleft containing the helix-turn-helix domain, as proposed by Berger *et al*. (1996). (d) and (e) Schematic representation and stereo views of the models in G2 geometry. The enzyme is bound to a left-handed DNA crossover. The 2-fold axis of the protein dimer corresponds with the 2-fold axis (a3) of the cross. The crystal structure of the 92 kDa fragment of yeast type II DNA topoisomerase which contains the residues 410–1202 of the 1429 residues of the polypeptide chain (Berger *et al*., 1996), as well as the crystal coordinates of right and left-handed DNA crossovers (se the legend to Figure 1) were used for modelling the ternary complexes. The docking procedure was performed using the program FRODO (Jones, 1978). Without sufficient information for modelling the structural changes occuring in the two partners upon binding, the protein and DNA crossovers were considered as rigid blocks. Knowing that DNA bending and important structural rearrangements in the protein structure could occur, we have estimated that stereochemical refinement and energy minimization would not improve significantly our models at the present state of the study. Symmetric ternary complexes are only obtained if the 2-fold symmetry axes of the protein dimer and of the crossover are colinear. The protein is then rotated around, and translated along the common symmetry axis, relatively to the DNA crossover for obtaining reasonable solutions according stereochemical criteria. The crystal coordinates of the DNA duplex of the CAP-DNA complex were used for modelling bent DNA segments (Schultz *et al*., 1991).

Table 2. Potential DNA domains and basic residues of yeast topoisomerase II proposed for interacting with DNA crossovers in each model

Model	Subunit	Subfragment	Secondary structure	Residues
G1	I	Bι	β1-β2	Arg419, Arg422, **Lys438**
			α7	Lys586, Lys594
			α8	**Arg622**, Lys625
	II	A′	α2-β1-α3	**Lys712**, **Lys713**, Lys716, **Lys720**
			β4-β5	Lys804, Lys811
			α14	**Lys1007**
			β17-β18	Lys1062, Lys1065
G2	N-gate double helix			
	I or II	A′	disordered linker	
			α2-β1-α3	**Lys712**, **Lys713**, Lys716
			β4-β5	Lys811
	C-gate double helix			
	I or II	A′	α8-β4	Lys804
			β14-β15	Lys983
			α14	**Lys1007**, Arg1015, **Lys1022**
			β17-β18	**Lys1062**, **Lys1065**,
			α17	Arg1120

Residues strictly and partially conserved among eukaryotic enzymes are represented with bold and underlined characters, respectively (Caron & Wang, 1993). Residues of the yeast enzyme that are not conserved are written is plain text; residues that are involved in the stabilization of the tertiary structure of the enzyme are not considered. The numbering scheme and nomenclature of structural domains is according to Berger *et al*. (1996) and corrected according Li & Wang (1997).

Potential DNA-binding domains

Although both G1 and G2 geometries are compatible with the mode of DNA binding proposed by Berger *et al*. (1996), the capture of DNA crossovers implies that the DNA segments interact with additional domains within the central hole of the enzyme. Many of them are identified as potential DNA-binding domains on the basis of their structural similarity with DNA binding motifs found in the literature.

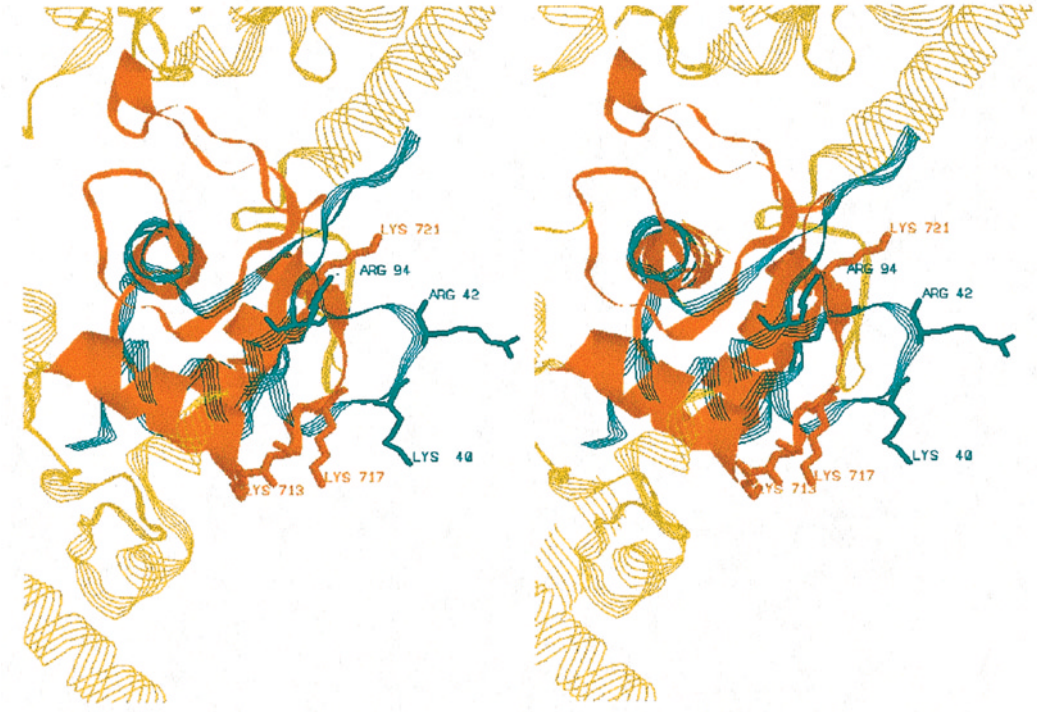

Figure 3. Structural analogy between the second DNA-binding domain of histone H5 and the winged-HTH domain of yeast topoisomerase H and spatial correspondence of clusters of basic residues involved in DNA binding. Stereo view of the superimposed helix-turn-helix domains of histone H5 (blue) and yeast type II DNA topoisomerase (orange). The surrounding domains of the yeast enzyme are represented in yellow. The recognition helices of the HTH domains are perpendicular to the plane of the Figure. This view shows the correspondence of the second DNA-binding domain of histone H5 containing the conserved Lys40, Arg42, Lys52 and Arg94 with the A′α2-β1 domain of the enzyme containing the cluster of lysine residues (Lys712, -713, -716 and -720) pointing at the top of the central hole (Right-hand side of the Figure).

β4-β5, β17-β18, α14. In the A′ subfragment, two sets of antiparallel β-sheets and an α-helix consisting of β4-β5, β17-β18 and α14 delimit a claw which could grip the DNA segments by interacting with the backbone and the major groove (Figure 2(b) and (e)). Several basic residues conserved among eukaryotic topoisomerase II sequences (Caron & Wang, 1993) are proposed for contacting the DNA segments (Table 2). DNA recognition motifs with antiparallel β-sheets were previously found in many transcriptional regulatory proteins such as the *Met* (Somers & Phillips, 1992) and the *Arc* (Raumann *et al*., 1994) repressor-operator complexes and the *Tus-Ter* complex (Kamada *et al*., 1996). In these complexes, the recognition sheets are inserted into the major groove and make extensive contact with the bases. Antiparallel β-sheets can also interact with the minor groove as found in the TBP/TATA-box complex (Kim, *et al*., 1993; Kim, J. L. *et al*., 1993) and recently in the IHF-DNA complex (Rice *et al*., 1996).

A second DNA-binding site in the HTH motif. A cluster of basic residues consisting of Lys712, Lys713, Lys716 and Lys720 located on a loop of the winged helix-turn-helix (HTH) domain points at the top of the central hole. These residues could also play a role in DNA recognition in both G1 and G2 models (Table 2, Figure 2(b) and (e)). A similar cluster of basic residues consisting of Lys40, Arg42, Lys52 and Arg94 was identified as a second DNA-binding domain in the winged-HTH motif of histone H5 (Ramakrishnan *et al*., 1993). The superimposition of the C^{α} atoms of the winged-HTH motif of H5 histone (blue) and of topoisomerase II (orange; RMS = 2.0 Å) brings the second DNA-binding site of histone H5 in close proximity to the loop of topoisomerase II in such a manner that the clusters of basic residues of the two proteins correspond to each other (Figure 3). Knowing that linker histones H5 or H1 recognize DNA crossovers (Krylov *et al*., 1993), it is tempting to speculate that this structural similarity reflects a similar DNA binding property of the enzyme. This cluster of basic residues is indeed conserved among eukaryotic topoisomerase II sequences (Caron & Wang, 1993).

Antiparallel coiled-coils. Antiparallel two-stranded coiled-coils were found in many different nucleic acid binding proteins (Lupas, 1996). Coiled-coils provide the docking site for tRNA in serine tRNA synthetase (Biou *et al*., 1994), and make multiple contacts with the DNA backbone in the serum response factor core (Pellegrini *et al*., 1995) and in the Klenow fragment (Beese *et al*., 1993) complexed with DNA. In both G1 and G2 solutions, the antiparallel coiled-coil formed with α14/α18 helices and the forked helical cradle formed by α19 could be involved in the gripping of the DNA segments in a similar manner. In agreement with this hypothesis α18 has a very basic character conserved among the other eukaryotic type II DNA topoisomerases (Caron & Wang, 1993; Table 2).

Symmetry and chirality in DNA crossover-topoisomerase II recognition

Rules for forming symmetric ternary complexes

Here, an analysis of the modes of assembly of yeast topoisomerase III (Berger *et al*., 1996) on right and left-handed tight DNA crossovers has been performed using the crystal coordinates of both partners. Our approach is, however, limited, since the DNA crossovers and the enzyme were considered as rigid blocks. Important structural rearrangements should occur in both the substrate and the enzyme upon binding and, as exemplified by the recent work by Morais Cabral *et al*. (1997), DNA topoisomerases II can adopt multiple conformations. Our analysis constitutes, therefore, a first attempt at understanding how topoisomerase II could form symmetric ternary complexes and shows that two solutions are possible, depending on the chirality of the crossover (Table 1). In the G1 solution, the 2-fold axis of the protein dimer is colinear with the 2-fold axis (a1) which bisects the large angle of a right-handed DNA crossover (Figure 2(a) to (c)). While the two DNA segments are involved in equivalent DNA-protein interactions, each individual DNA double helix interacts asymmetrically with the enzyme with respect to the 2-fold axis of the topoisomerase II homodimer (Figure 2(a) and (b)). In the G2 solution, the 2-fold axis of the protein dimer corresponds with the 2-fold axis (a3) normal to the plane of a left-handed crossover (Figure 2(d) and (e)). Each DNA segment has its own mode of recognition and is involved in different DNA protein contacts. In contrast, the double helices are symmetrically recognized around their dyad axes, regarding the 2-fold axis of the protein dimer, in the classical manner found in many structures of protein bound to a palindromic DNA sequence.

Alternative modes of DNA binding

G1 and G2 geometries are both consistent with footprinting experiments which show that topoisomerase II can protect a region of 25 nucleotides (Lee *et al*. 1989b; Alsner *et al*., 1996). If the DNA segments are bent into the positive cleft for contacting the HTH motif (Figure 2(c)), their interactions with the antiparallel β-sheet claws, the cluster of lysine residue located on α2-β1-α3 and the HTH motif of the A′ subfragment could roughly correspond to the three distinct regions of contact proposed by Alsner *et al*. (1996). The important solvent accessibility along the duplexes within the models fits well with the lack of protection against methylation (Lee *et al*., 1989b). The identification of potential DNA-binding domains which could establish extensive interactions with the DNA crossovers provide further support to our

models. Moreover, the structural analogy between the second DNA-binding site of histone H5 and a loop containing a cluster of conserved basic residues in topoisomerase II is particularly relevant, knowing that histone H5 recognizes DNA crossovers (Figure 3). This finding predicts that replacing lysine residues (712, 713, 716 and 720) by neutral or acid residues using site-directed mutagenesis would probably alter the binding of crossover and the enzymatic activity. The other residues listed in Table 2 are candidates for such experiments in order to test the reliability of the models.

Symmetry and sequence recognition

In contrast with other homodimeric enzymes that recognize palindromic sites, DNA topoisomerase II binds asymmetric DNA sequences (Sander & Hsieh, 1985; Lee *et al*., 1989a,b; Osheroff *et al*., 1991), discriminates the two strands of a double helix, and cleaves one preferentially to the other (Muller *et al*., 1988; Andersen *et al* 1989; Zechiedrich *et al*., 1989). The G1 geometry which displays an asymmetric disposition of each DNA segment with respect to the 2-fold axis of the protein dimer is consistent with these observations. In addition, it suggests how the protein domains located in the central hole could contribute to the recognition of the DNA sequence in a position remote from the cleavage site. In contrast, the G2 geometry implies the recognition of a palindromic DNA sequence.

Symmetry and chirality: a mode of discrimination of tight DNA nodes?

This study also reveals that topoisomerases II can discern the chirality of tight DNA crossovers on the basis of their structural properties. Right-handed crossovers differ from left-handed DNA crosses by their geometry and symmetry (Table 1). It is therefore possible that the difference of symmetry could provide a mechanism for discriminating chirality. The G1 geometry is compatible with tight binding of right-handed crossovers while the G2 geometry is compatible with the symmetric binding of left-handed crosses. This mode of selection could provide some insights for understanding how topoisomerase II recognize preferentially positive (right-handed) nodes (Shaw & Wang, 1997).

Recognition of DNA crossovers: functional considerations

DNA-DNA interactions and structural transitions

Crystallographic studies of DNA duplexes have shown that close DNA-DNA interactions can induce the destabilization of DNA secondary structure in specific sequences called ''compaction responsive sequences'' such as $(CA)_n$ or related sequences (Timsit & Moras, 1991, 1995, 1996; Y. Timsit *et al*., unpublished). It is interesting to note that topoisomerase II recognizes and preferentially cleaves DNA at very similar sequences (Sander & Hsieh, 1985, Spitzner & Muller, 1988; Spitzner *et al*., 1989). One hypothesis could be that the structural changes induced at such sequences by the close DNA-DNA interactions occuring within the ternary complex participate in the enzymatic activity. This could help us to understand why the binding of a second helix can enhance the enzymatic cleavage of a DNA segment (Corbett *et al*., 1992) and suggests that the loose consensus for topoisomerase II cleavage reflects the requirement for a sequence ability to melt upon DNA-DNA close association.

Strand-passage and strand exchange reactions

The models of ternary complexes described here can be thought as intermediates of the strand-passage reaction or intermediates of the recombinase-like activity of the enzyme. They could be also related to the structural role of the protein. In the current mechanisms proposed for the strand-passage reaction, the binding of the G (gate) DNA segment precedes the binding of the T (transported) segment (Berger *et al*., 1996; Figure 4(a)). It is thought that the crystal forms of the gyrase and the yeast enzyme represent two steps of the reaction corresponding to the binding of the G segment, before and during the strand-passage, respectively (Berger *et al*., 1996; Morais Cabral *et al*., 1997). However, in these studies the G segment is modelled into a cleft having a positive potential located outside the central hole. This geometry seems difficult to reconcile with the observation that a linear DNA segment thread through the central hole can be cleaved by the enzyme (Roca & Wang, 1992; Chen & Liu, 1994; Maxwell, 1996).

Here, an alternative view in which tight DNA crossovers are bound into the central hole before strand cleavage is investigated. Indeed, it was previously suggested that the open conformation of the enzyme could capture two DNA segments which would be then stored within the central hole after ring closure (Roca & Wang, 1992; Chen & Liu, 1994; Maxwell, 1996). In addition, the role of topoisomerase II during the anaphase (Sikorav *et al*.,1998) implies that the enzyme interacts with tight DNA crossovers before the cleavage of the G segment. The G2 solution could correspond to a pre-strand passage ternary complex occuring before the cleavage of the G segment (Figure 4(b)). The reaction could then take place within a closed configuration of the enzyme. Indeed, two experiments suggest that the enzyme can catalyze the DNA cleavage in its closed conformation, in the presence of AMP-PNP (Roca & Wang, 1992) or in the absence ATP or analogue (Corbett *et al*., 1992). The release of the DNA segments after the strand-passage reaction could occur in a one-gate or in a two-gate mechanism. Alternatively, the G2 solution could correspond to a post-strand cleavage

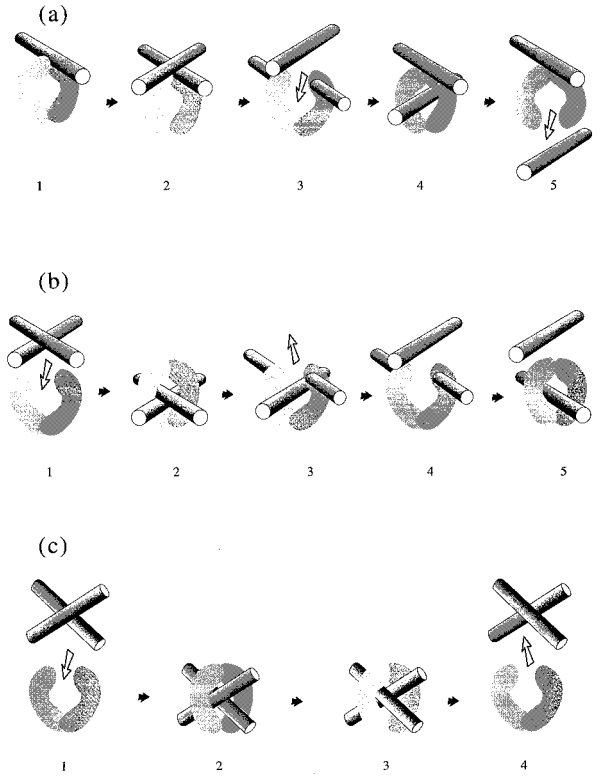

Figure 4. Modes of DNA crossover recognition and strand-passage mechanisms. (a) Simplified representation of the two-gate mechanism for strand-passage proposed by Berger *et al*. (1996). (b) A hypothetical one-gate mechanism for strand passage reaction compatible with binding of a crossover as in the G2 solution. (c) A hypothetical one-gate mechanism for strand-passage reaction and illegitimate recombination compatible with the G1 solution. This mechanism assumes the existence of recombination-like DNA intermediates. An incomplete reaction is expected to generate recombined molecules.

complex, when the G segment has been resealed and prior the release of the T segment.

In G1 geometry, the mode of DNA binding is more appropriate for a recombinase-like mechanism. A single-strand cleavage could occur on each DNA segment which contacts the active site tyrosine residues of the two monomers. This situation resembles to that of site-specific recombination and could lead to the inversion of the chirality of the DNA crossover trapped within the topoisomerase ring, assuming the occurrence of recombination-like intermediates such as transient fourway junctions (Figure 4(c)). This hypothesis, which suggests an alternative pathway for solving the topological problem of strand-passage, is consistent with the observation of single-strand cleavages (Muller *et al*., 1988, Lee *et al*., 1989a), the implication of the enzyme in illegitimate recombination (Sperry *et al*., 1989), in SV40 integration (Bodley *et al*. 1993) and its ability to perform intermolecular ligation (Gale & Osheroff, 1992; Schmit *et al*., 1994). Following this view, the products of illegitimate recombination reaction catalysed by type II DNA topoisomerases could be understood as the intermediates of a normal but incomplete pathway of the enzymatic reaction.

Conclusion

The present study has shown that for forming symmetric ternary complexes between topoisomerase II and tight DNA crossovers, two geometric solutions are possible, depending on the chirality of the crosses. Each solution, which is consistent with different sets of experimental data of the literature, could correspond to different functions of the enzyme. This work provides structural insights for better understanding the role of chirality and symmetry in topoisomerase II-DNA crossover recognition, suggests testable experiments to further elucidate the structure of ternary complexes, and raises new questions about the relationships between the mechanism of strand-passage and strand exchange catalyzed by the enzyme.

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